

Dkt. 53801/JPW

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Nika Adham, et al.
Serial No.: 09/116,676 Examiner: G. Draper
Filed: July 16, 1998 Group Art Unit: 1647
For: DNA ENCODING A HUMAN OB RECEPTOR (OB-RE) AND
USES THEREOF

1185 Avenue of the Americas
New York, New York 10036

#12
BGS
7/6/9

Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

**DECLARATION OF NIKA ADHAM, BETH BOROWSKY,
NIGEL LEVENS, AND RADEK C. SKODA
UNDER 37 C.F.R. §1.131**

We, Nika Adham, Beth Borowsky, Nigel Levens, and Radek C. Skoda, hereby declare as follows:

1. We conceived of the invention claimed in the above-identified patent application, i.e., a process for determining whether a chemical compound specifically binds to a soluble polypeptide with specific properties as recited in claim 224 as amended in the Amendment filed concurrently with the filing of the Declaration (the "Binding Assay").

2. Prior to December 31, 1996, a Binding Assay was performed by Noel Boyle under the direction and supervision of coinventor Nika Adham in the United States at the laboratories of Synaptic Pharmaceutical Corporation, an assignee of record of the subject application. Copies of pages 189 and 190 of Noel Boyle's notebook number 11914 and pages 2-7 of Noel Boyle's notebook number 11915 detailing the performance of such a Binding Assay for determining whether leptin specifically binds to human Ob-Re, a soluble polypeptide as recited in claim 224 are attached hereto as **Exhibit A**. Although the dates have been redacted from these

EXHIBIT 2

Nika Adham, et al.
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Page 2

notebook pages, all dates are prior to December 31, 1996. Thus, at least one embodiment of the invention claimed was reduced to practice in the United States prior to December 31, 1996.

3. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1/29/01
Date

1/30/01
Date

5/18/01
Date

4/9/01
Date

Nika Adham
Nika Adham

Beth Borowsky
Beth Borowsky

N. Levens
Nigel Levens

R. Skoda
Radek C. Skoda

Project N

Book No.

TITLE

Leptin Soluble membranes

2

D: 125-I

Fr m Page USER: 3

COMMENT:

PRESET TIME : 1.00
DATA CALC : CPM H# : NO SAMPLE REPEATS: 1 PRINTER
COUNT BLANK : NO IC# : YES REPLICATES : 1 RS232
TWO PHASE : NO AOC : NO CYCLE REPEATS : 1
SCINTILLATOR: LIQUID LUMEX: NO LOW SAMPLE REJ: 0
LOW LEVEL : NO HALF LIFE CORRECTION DATE: none

ISOTOPE 1: 125I XERROR: 2.00 FACTOR: 1.000000 BKG. SUB:

SAM NO	POS	TIME MIN	IC#	125I CPM	XERROR	LUMEX %	ELAPSED TIME
1	**1	1.00	4.785	2278.01	4.19	0.01	1.29
2	**2	1.00	5.337	762.01	7.25	0.02	2.62
3	**3	1.00	4.913	2074.06	4.39	0.01	3.97
4	**4	1.00	4.881	1816.07	4.69	0.01	5.34
5	**5	1.00	4.830	1701.08	4.85	0.01	6.67
6	**6	1.00	4.737	12115.13	4.35	0.01	8.02
7	**7	1.00	4.802	1761.13	4.77	0.01	9.39
8	**8	1.00	5.166	1416.12	5.31	0.01	10.74
9	**9	1.00	5.067	11375.13	5.39	0.01	12.09
10	**10	1.00	5.319	823.09	6.97	0.02	13.45
11	**11	1.00	5.427	749.09	7.31	0.03	14.81
12	**12	1.00	5.166	661.08	7.78	0.03	16.15
13	**13	1.00	4.810	2520.34	3.98	0.00	17.52
14	**14	1.00	5.642	688.10	7.62	0.02	18.87
15	**15	1.00	4.991	1902.30	4.59	0.01	20.22
16	**16	1.00	4.869	1859.31	4.64	0.01	21.60
17	**17	1.00	5.028	1882.34	4.61	0.01	22.96
18	**18	1.00	4.901	1900.36	4.59	0.01	24.30
19	**1	1.00	4.927	2042.41	4.43	0.01	25.77
20	**2	1.00	5.037	1492.32	5.18	0.01	27.12
21	**3	1.00	5.180	1179.26	5.82	0.01	28.47
22	**4	1.00	5.407	930.22	6.56	0.02	29.85
23	**5	1.00	5.645	777.19	7.17	0.03	31.20
24	**6	1.00	5.477	833.21	6.93	0.01	32.55
25	**7	1.00	4.896	2788.74	3.79	0.01	33.92
26	**8	1.00	5.175	2653.74	3.88	0.01	35.27
27	**9	1.00	4.842	2310.67	4.16	0.01	36.62
28	**10	1.00	4.901	2386.72	4.09	0.00	38.01
29	**11	1.00	4.972	2022.63	4.45	0.01	39.35
30	**12	1.00	4.852	1964.63	4.51	0.01	40.71
31	**13	1.00	5.096	2824.94	3.76	0.01	42.07
32	**14	1.00	5.028	2105.72	4.36	0.01	43.42
33	**15	1.00	5.034	1995.71	4.48	0.01	44.77
34	**16	1.00	5.152	1807.66	4.70	0.01	46.16
35	**17	1.00	5.238	1874.70	4.62	0.01	47.51
36	**18	1.00	4.993	2026.78	4.44	0.01	48.85
37	**1	1.00	5.635	787.31	7.13	0.02	50.32
38	**2	1.00	5.818	789.32	7.12	0.01	51.67
39	**3	1.00	5.614	682.29	7.66	0.02	53.02
40	**4	1.00	5.521	806.35	7.04	0.02	54.40
41	**5	1.00	5.520	660.29	7.78	0.02	55.75
42	**6	1.00	5.739	718.32	7.46	0.02	57.10
43	**7	1.00	5.784	724.34	7.43	0.01	58.47
44	**8	1.00	5.552	725.34	7.43	0.02	59.82
45	**9	1.00	5.613	725.35	7.43	0.02	61.17
46	**10	1.00	5.828	587.29	8.25	0.02	62.55

ObRe
Lept -4ObRe
Lept -4T₂ 2230

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EXHIBIT A

Nika Adham, et al.

Serial No.: 09/116,676

Filed: July 16, 1998

Leplin soluble membranes

TITLE	SAM	POS	TIME	IC#	125I	LUMEX	ECLIPSED
Fr m P	NO		MIN		CPM	% ERROR	TIME
	47	**11	1.00	5.951	790.40	7.12	63.90
	48	**12	1.00	5.917	696.36	7.58	65.25
	49	**13	1.00	5.789	456.24	9.37	66.64
	50	**14	1.00	5.282	848.46	6.87	67.97
	51	**15	1.00	5.938	523.29	8.75	69.34
	52	**16	1.00	5.143	551.31	8.52	70.70
	53	**17	1.00	5.821	517.30	8.80	72.05
	54	**18	1.00	5.782	542.32	8.59	73.40
	55	**1	1.00	6.896	577.34	8.33	74.89
	56	**2	1.00	6.307	428.26	9.67	76.24
	57	**3	1.00	5.909	533.33	8.66	77.59
	58	**4	1.00	5.996	493.31	9.01	78.95
	59	**5	1.00	6.075	521.33	8.76	80.30
	60	**6	1.00	5.425	345.22	10.77	81.65
	61	**7	1.00	5.346	684.45	7.65	83.04
	62	**8	1.00	6.001	757.51	7.27	84.39
	63	**9	1.00	5.880	755.51	7.28	85.72
	64	**10	1.00	5.927	788.55	7.12	87.10
	65	**11	1.00	5.825	901.63	6.66	88.45
	66	**12	1.00	5.973	797.57	7.08	89.81
	67	**13	1.00	6.264	807.58	7.04	91.19
	68	**14	1.00	6.368	765.56	7.23	92.54
	69	**15	1.00	6.138	801.60	7.07	93.89
	70	**16	1.00	6.385	766.58	7.23	95.25
	71	**17	1.00	6.255	787.60	7.13	96.60
	72	**18	1.00	5.925	830.65	6.94	97.95
	73	**1	1.00	6.061	732.58	7.39	99.42
	74	**2	1.00	5.934	738.59	7.36	100.77
	75	**3	1.00	5.994	703.57	7.54	102.12
	76	**4	1.00	5.710	657.54	7.80	103.50
	77	**5	1.00	6.291	503.42	8.92	104.85
	78	**6	1.00	6.667	456.39	9.37	106.20
	79	**7	1.00	6.552	466.40	9.26	107.57
	80	**8	1.00	6.639	494.43	9.00	108.92
	81	**9	1.00	6.056	860.75	6.82	110.27
	82	**10	1.00	5.902	726.65	7.42	111.65
	83	**11	1.00	6.462	808.73	7.04	113.00
	84	**12	1.00	6.495	644.59	7.88	114.35
	85	**13	1.00	6.222	771.71	7.20	115.72
	86	**14	1.00	6.174	739.69	7.36	117.07
	87	**15	1.00	6.219	644.61	7.88	118.42
	88	**16	1.00	6.132	540.52	8.61	119.80
	89	**17	1.00	6.709	394.38	10.08	121.15
	90	**18	1.00	6.737	545.53	8.57	122.50
	91	**1	1.00	6.458	624.62	8.01	123.97
	92	**2	1.00	6.216	568.57	8.39	125.32
	93	**3	1.00	6.198	728.73	7.41	126.67
	94	**4	1.00	6.151	763.78	7.24	128.05
	95	**5	1.00	6.245	883.91	6.73	129.40
	96	**6	1.00	6.383	787.82	7.13	130.75
	97	**7	1.00	6.123	5482.76	2.70	132.12
	98	**8	1.00	6.846	916.97	6.61	133.47
	99	**9	1.00	6.422	5008.37	2.83	134.82
	100	**10	1.00	6.311	4978.39	2.84	136.21
	101	**11	1.00	6.532	4710.15	2.92	137.56
	102	**12	1.00	6.668	4465.94	2.99	138.91
	103	**13	1.00	6.867	3962.42	3.18	140.27
	104	**14	1.00	6.903	2948.32	3.69	141.62

Mod

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Mod

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Loglin Soluble membranes

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SAM NO	POS	TIME MIN	IC#	125I CPM	%ERROR	LUMEX %	ELAPSED TIME
105	**15	1.00	6.749	1720.96	4.82	0.01	142.99
106	**16	1.00	6.994	1274.46	5.61	0.02	144.34
107	**17	1.00	7.078	1013.17	6.27	0.01	145.70
108	**18	1.00	6.841	989.16	6.36	0.01	147.05
109	**1	1.00	6.536	3233.82	3.52	0.00	148.54
110	**2	1.00	6.808	862.03	6.82	0.02	149.89
111	**3	1.00	6.791	4667.62	2.93	0.00	151.24
112	**4	1.00	6.839	3698.49	3.29	0.00	152.61
113	**5	1.00	6.664	4233.19	3.08	0.00	153.96
114	**6	1.00	6.871	3384.18	3.44	0.00	155.31
115	**7	1.00	6.886	3795.73	3.25	0.00	156.69
116	**8	1.00	7.171	2439.07	4.05	0.00	158.04
117	**9	1.00	7.104	1767.24	4.76	0.01	159.39
118	**10	1.00	6.970	1076.38	6.10	0.01	160.76
119	**11	1.00	7.187	864.11	6.81	0.01	162.10
120	**12	1.00	6.686	984.28	6.38	0.02	163.46
121	**13	1.00	6.759	5354.02	2.74	0.00	164.84
122	**14	1.00	6.855	4320.72	3.04	0.00	166.19
123	**15	1.00	6.895	3913.22	3.20	0.00	167.54
124	**16	1.00	6.852	4462.00	3.00	0.00	168.91
125	**17	1.00	6.848	4448.03	3.00	0.00	170.26
126	**18	1.00	6.895	4898.69	2.86	0.00	171.61
127	**1	1.00	6.836	5364.39	2.73	0.00	173.09
128	**2	1.00	7.120	5314.38	2.75	0.00	174.44
129	**3	1.00	6.866	4718.60	2.91	0.00	175.79
130	**4	1.00	6.750	4763.72	2.90	0.00	177.16
131	**5	1.00	6.787	4473.36	2.99	0.00	178.51
132	**6	1.00	6.598	5404.74	2.72	0.00	179.86
133	**7	1.00	7.420	963.39	6.45	0.01	181.24
134	**8	1.00	7.475	847.23	6.88	0.02	182.59
135	**9	1.00	7.183	865.27	6.80	0.02	183.94
136	**10	1.00	7.585	809.19	7.04	0.02	185.30
137	**11	1.00	7.372	835.24	6.93	0.02	186.65
138	**12	1.00	7.245	905.36	6.65	0.02	188.01
139	**13	1.00	7.717	829.25	6.95	0.01	189.39
140	**14	1.00	7.334	823.25	6.98	0.01	190.74
141	**15	1.00	7.186	878.34	6.75	0.01	192.09
142	**16	1.00	7.266	807.24	7.04	0.01	193.45
143	**17	1.00	7.436	993.54	6.35	0.01	194.80
144	**18	1.00	6.908	1018.59	6.27	0.01	196.15
145	**1	1.00	6.312	2363.72	4.12	0.00	197.64
146	**2	1.00	6.683	803.27	7.06	0.01	198.99
147	**3	1.00	6.538	2331.72	4.15	0.01	200.34
148	**4	1.00	6.341	2644.25	3.89	0.00	201.70
149	**5	1.00	6.488	2583.18	3.94	0.00	203.06
150	**6	1.00	6.486	2725.44	3.83	0.01	204.41
151	**7	1.00	6.632	2159.54	4.31	0.01	205.79
152	**8	1.00	6.725	1890.12	4.60	0.01	207.14
153	**9	1.00	6.534	1441.39	5.27	0.01	208.49
154	**10	1.00	6.915	949.59	6.50	0.01	209.85
155	**11	1.00	6.659	804.35	7.06	0.02	211.20
156	**12	1.00	6.557	774.31	7.16	0.01	212.55
157	**13	1.00	6.619	2376.05	4.11	0.01	213.94
158	**14	1.00	6.603	796.37	7.09	0.01	215.29
159	**15	1.00	6.418	2617.52	3.91	0.00	216.64
160	**16	1.00	6.540	2429.22	4.06	0.00	218.00
161	**17	1.00	6.346	2521.41	3.99	0.00	219.36
162	**18	1.00	6.273	2504.40	4.00	0.01	220.71

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Optic obse

OB-R₂
optical
medium

T=4780

M=881

Regule med
Obse

Lept P

Regule med
Obse

Lept

Project N _____
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TITLE SPA LGPT in Soluble Membranes.

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SPA -

Auerokan kit
proximity assay

Plate 1 - hOB-Re ++

Plate 2 - hOB-Re ++ mock

Plate 3 - [Cos 7] hOB-Re, # B025 P91 (optimum medium) 1:2

Plate 4 - [Cos 7] hOB-Re, # B025 P91 (normal medium) 1:2

Plate 5 - [Cos 7] hOB-RB, # B07 P55

1ml = 2.4 (1:3)

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TITLE SPALEPTIN SOLUBLE MEMBRANES

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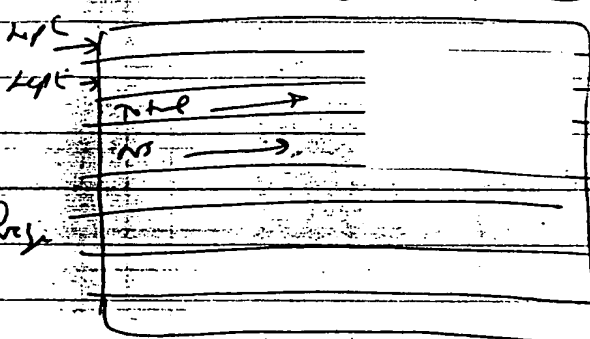
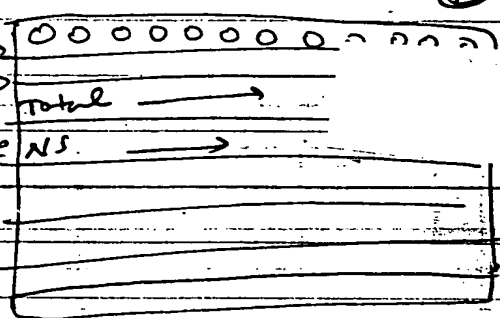
SPA binding on Oble -

Assay buffer: use leptin binding assay buffer + 1% N.S.A.

Oble (medium) ①

Mock (medium) ②

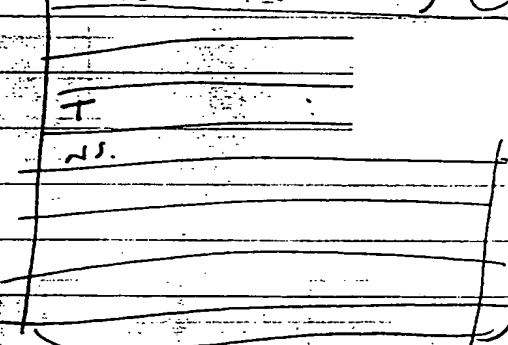
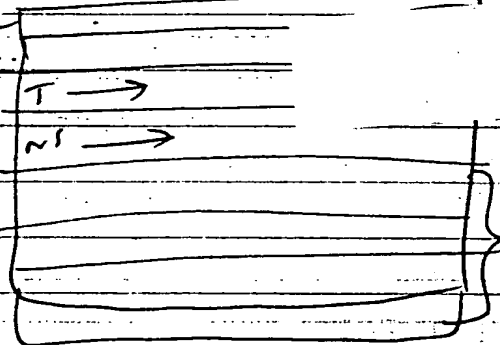
re-SPA curve
leptin curve
no dry
no dry



Oble (optimum) ③

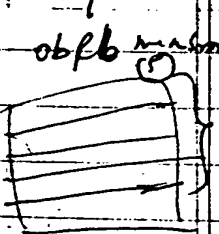
Oble (medium) ④

leptin curve
leptin curve

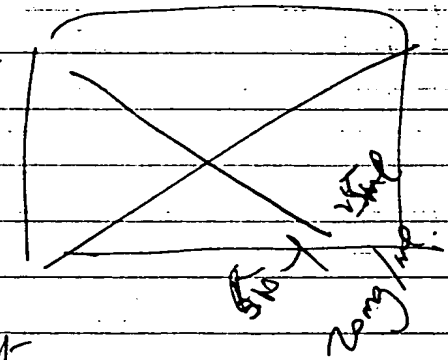


Dilution
1:3

obfb membrane



control membrane
as usual Membr



100%
2% lipid
2% Dye or total

Add to lipid beads

run lamp other brought

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TITLE Leptin Via Scintillation Proximity Assay Pr j c

189

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Protocol #: 13
10:54

Count Time (minutes): 1.00
Assay Type: CPM
Background Substrate: IPA 3kg
Outlier: 5.0 FLAG
Screening: OFF

Window A
Nuclide: I-125
Half Life (hours): 0.00
Multiplier: 1.0000
MCV Flag Limit: 0.00

Leptin
WARNING: BACKGROUND DAT

S# A:CPM A:ZERR B:CPM B:ZERR
1 123406 0.28 0.0
3 MISSING TUBE(S)

Table 1. Assay protocol

	Non-specific binding (NSB)	Zero standard (B ₀)	Standards	Test	Solvent Control
Buffer	-	50μl	-	-	-
Standards	50μl	-	50μl	-	-
Test Compound	-	-	-	50μl	-
Tracer	50μl	50μl	50μl	50μl	50μl
Test Solvent*	-	-	-	-	50μl
Cell preparation	50μl	50μl	50μl	50μl	50μl
WGA SPA beads	50μl	50μl	50μl	50μl	50μl

Cap tubes, shake overnight (16-24hr) at room temperature (15-30°C)
Leave to settle for 1 hour

Count in scintillation counter

* For example in organic solvents add the relevant solvent

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$[^{125}\text{I}]$ Leptin - 0.1 nM (F) 100,000 cpm/μl

Dry plate

Non-specific 1×10^{-6} M (B) cold leptin.

MAKER 8 ml (^{125}I) Leptin

58.9 μCi / 0.1 ml 1840 Ci/mole

$$\frac{58.9 \mu\text{Ci} / 0.1 \text{ ml} \times 1.28 \text{ F}}{1,840 \times 10^9 \mu\text{Ci/mole}} = \frac{169}{1,840 \times 10^9} = 9.19 \times 10^{-8} \frac{\text{mole}}{\text{ml}}$$

$$9.19 \times 10^{-8} \frac{\text{mole}}{\text{ml}} \cdot V = 1 \times 10^{-9} \frac{\text{mole}}{\text{ml}} \cdot 8 \text{ ml}$$

$$V = 82 \mu\text{l}$$

To Page _____

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Date _____

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